MODIFICATION OF LIGNIN COMPOSITION OF GYMNOSPERMS

TECHNICAL FIELD

This invention relates to the modification of the lignin composition of gymnosperm species, particularly conifer trees, to make such species more suitable for commercial exploitation.

BACKGROUND ART

Lignin is a cell wall component present in vascular plants that decreases the permeability of cells, contributes to the strength and rigidity of the stem, and protects microfibrils from chemical, physical, and biological attack (Bugos et al. 1991 [4]). [*Note: for full details of references mentioned herein, see the section below headed REFERENCES, the numbers provided in square brackets corresponding to the numbers in that section.*] Despite its advantage to the plant, lignin greatly affects the agro-industrial uses of plants. Lignin content and composition alter the digestibility and dietary conversion of herbaceous crops and are undesirable in the conversion of wood into paper and pulp (Campbell and Sederoff 1996 [6]). Although lignin can contribute up to 25% of the mass of wood, from a pulp and paper viewpoint, lignin does not contribute to the usable biomass in pulping and hence is waste. More importantly, the extraction of lignin during chemical pulping is a costly and difficult process, involving chemical removal. There is a negative correlation between the amount of lignin removed and fiber yield with chemical pulping. Therefore, because the removal of lignin from fibers is a major cost, the modification of both lignin content and composition is a major focus of several research establishments world wide. Of importance is that trees with altered lignin, either decreased content or modified composition to reduce the energy needed to extract the lignin, could allocate more resources to the production of pulpable biomass with decreased costs.

Chemically, lignin is a highly complex network of phenylpropanoid units derived from the oxidative polymerization of one or more of three monolignol precursors which are the end products of the three major branches of the phenylpropanoid pathway (as shown in Figure 1 of the accompanying drawings, introduced the section below headed BRIEF DESCRIPTION OF THE DRAWINGS). As shown in the figure, branch 1 of the pathway yields the monolignol ρ-coumaryl alcohol which makes up the ρ-hydroxyphenyl residue when polymerized into lignin and is present in both angiosperms and gymnosperms. Branch 2 yields the monolignol coniferyl alcohol which makes up the guaiacyl residues when polymerized into lignin and is present in both angiosperms and gymnosperms, yet is the predominant monolignol in gymnosperms. Branch 3 yields sinapyl alcohol which makes up the syringyl residues when polymerized into lignin and is present only in angiosperms, with very few exceptions. These exceptions include reports of syringyl lignin in the gymnosperm species *Podocarpus* and in some species of the *Gnetales*. However, these exceptions are considered rare and are usually not even mentioned in reviews on lignin biosynthesis.

The presence of syringyl residues in angiosperm lignin via branch 3 in the phenylpropanoid pathway accounts for angiosperm lignin being easier to remove during pulping than gymnosperm lignin. One reason syringyl-lignin is easier to remove during pulping, as compared to guaiacyl-lignin produced by gymnosperms, is that the C-5 carbon of the phenyl ring in syringyl-lignin is protected by methoxylation from forming a C5-C5 bond with adjacent monolignol phenyl rings. Once formed, this C-C bond is very difficult to break during delignification and the presence of these bonds accounts for the fact that gymnosperm lignin is harder to pulp than angiosperm lignin.

The inventors of the present invention theorized that if the phenylpropanoid pathway in gymnosperms could be modified such that gymnosperm plants could produce lignin containing syringyl residues, via branch 3, or a modification thereof, of the phenylpropanoid pathway, this would be of great benefit because significant reductions in the pulping costs associated with lignin removal in gymnosperms would be enabled.

However, this requires the creation of an entirely new pathway in gymnosperms, i.e., the creation of the enzymes and substrates in gymnosperm species to enable the branch 3 phenylpropanoid pathway synthesis of syringyl-lignin to proceed through to completion. This is quite different in concept from arranging for over-expression of a gene in an existing metabolic pathway, which is likely to shuttle more metabolites through the pathway, provided other steps do not become limiting.

There are numerous reports on the modification of the phenylpropanoid pathway by genetic engineering. One example is the "sense" suppression of PAL by a bean PAL2 gene in tobacco. These experiments demonstrated that PAL activity becomes rate-limiting to lignin deposition when levels are 3- to 4-fold lower than in wild-type plants (Bate et al. 1994 [2]). While PAL may hold promise for use in engineered lignin modification, it has been suggested that due to its key role in general phenylpropanoid metabolism, the interruption of PAL synthesis would also affect other biochemical pathways. In contrast, the activity of CAD, an enzyme well downstream in the lignin biosynthetic pathway, can be reduced to 10% of normal levels and still have no effect on the quantity of lignin, although clear qualitative differences are observed (Halpin et al. 1994 [12]). From these and other studies on the manipulation by genetic engineering of key enzymes in the lignin biosynthetic pathway (OMT (Dwivedi et al. 1994 [10]; Ni et al. 1994 [16]), F5H (Bell-Lelong et al. 1997, [3]), and peroxidase (Lagrimini et al. 1990

[14])), it is clear that lignin modification is possible. However, such studies also highlight how extremely difficult it is to achieve a change in lignin composition and how it is even more difficult to achieve a change that has commercial relevance. In the CAD antisense work, Halpin et al. [12] reported increased lignin extractability in only 2% of the transformed lines tested. In other words, 98% had no change despite morphological changes such as the appearance of red xylem.

Therefore, these disclosures do not specifically relate to techniques involving genetic engineering to create a lignin which is unique to the plants of interest, i.e. gymnosperms. Firstly, all the published work on the genetic engineering of plants for altered lignin has been done in angiosperms and was done to manipulate an existing endogenous enzyme and biochemical pathway. Even with this, the results were variable, and changing lignin parameters to a level such that they had commercial advantages was difficult. Secondly, the only example of lignin modification in gymnosperms where a gene for a specific enzyme in the phenylpropanoid pathway was down-regulated occurred in a naturally occurring mutant which had virtually no CAD activity (for a review see Whetton et al. 1998 [17]). In this case, genetic engineering was not used and the regulation was again dependent on natural mutation which altered the expression of an endogenous gene.

International patent application PCT/US96/20094, published on July 3, 1997 as WO 97/23599, in the name of Clint Chapple as inventor, and assigned jointly to E.I. Du Pont De Nemours and Company, and Purdue Research Foundation, discloses the nucleotide sequence of a gene encoding an F5H enzyme, the transformation of the genome of plants with the gene, and the resulting modification of lignin composition of the plants. The present application builds on this Chapple application and goes beyond, to describe

the use of this gene, either alone or in conjunction with other genes, to introduce a lignin biosynthetic pathway into gymnosperms.

DISCLOSURE OF THE INVENTION

An object of the invention is to modify gymnosperms by genetic engineering so that modified gymnosperm plants produce lignin of a type that differs from the lignin of wild-type plants of the same species and that is more easily accommodated in commercial utilization of such plants.

Another object of the invention is to modify the lignin precursors in gymnosperms to provide modified monolignol residues, and preferably, a greater content of syringyl residues, or other residues with a side group at the C-5 position of the monolignol ring.

According to one aspect of the present invention, there is provided a process of producing a transformed gymnosperm plant or plant precursor having a genome containing at least one expressible transgene that results in modification of lignin composition in the gymnosperm plant compared to an average lignin composition of untransformed wild-type plants of the same gymnosperm species, which process comprises: providing a vector containing at least one expressible transgene that results in modification of the lignin composition in the gymnosperm plant; introducing the vector into cells of a gymnosperm plant to produce transformed cells; regenerating transformed gymnosperm callus or shoots from the transformed cells; maturing embryos or plants from the transformed callus or shoots; and generating transformed plant embryos, seeds, seedlings or plants from the matured embryos.

Without wishing to limit the generality of meaning of the term "transgene", we should point out that the term is intended to include foreign DNA (transgenic or introduced genes) that is introduced into a genome of a gymnosperm plant.

According to another aspect of the invention, there is provided a transformed gymnosperm plant or plant precursor having a genome containing at least one expressible transgene that results in modification of lignin composition in the gymnosperm plant compared to an average lignin composition of untransformed wild-type plants of the same gymnosperm species.

Most preferably, the lignin of the transformed gymnosperm plant contains detectable syringyl residues, or other residues with a side group at the C-5 position of the monolignol ring, whereas the lignin of the wild-type plants contains no detectable syringyl residues or other residues with a side group at the C-5 position of the monolignol ring.

Preferably, the expressible transgenes are genes that code for enzymes required for the lignin biosynthetic pathway, and more preferably the third branch of the pathway by which branch 2 intermediates are converted to sinapyl alcohol. It is therefore to be noted that, in the present invention, at least in its preferred forms, gymnosperm plants are being genetically engineered with genes which encode at least one enzyme that is not normally present in these plants, thereby creating a branch to an existing pathway in gymnosperm plants. The invention therefore differs considerably from prior art procedures that have merely involved the modification of existing pathways in angiosperm plants utililizing enzymes already present in the wild-type plants.

Most preferably, the transgene(s) introduced into the gymnosperm plants includes a ferulate 5-hydroxylase gene, or a transgene that is substantially homologous to said ferulate 5-hydroxylase gene, or a transgene that has an equivalent function, either alone or in conjunction with other genes needed for the biosynthesis of a lignin, i.e. that results in a lignin composition containing syringyl residues. By a "gene that is substantially homologous to said ferulate 5-hydroxylase gene", we mean a gene which can be shown to have ferulate 5-hydroxylase activity in yeast or having at least 50% homology, and more preferably at least 75% homology, to the F5H gene while exhibiting an ability to modify the lignin content of the gymnosperm plant *in vivo*.

The ferulate 5-hydroxylase gene (or equivalent gene) either alone or in conjunction with other genes, are normally operably linked with at least one regulatory sequence, e.g. cauliflower mosaic virus 35S promoter, a promoter for a phenylalanine ammonia lyase gene, a promoter for a p-coumaryl CoA ligase gene, a promoter for cinnamate 4-hydroxylase or other plant promoters capable of controlling expression of plant genes.

The gymnosperm plants produced by the present invention are preferably from the order *coniferales*. Thus, they may be from the *Picea* species (e.g. *Picea glauca, Picea sitchesis*, or *Picea engelmanii*), or from the *Pinus* species (e.g. *Pinus taeda* or *Pinus radiata*).

According to another aspect of the invention, there is provided a biomass derived from a genetically transformed gymnosperm plant, said biomass containing lignin having syringyl residues, or other residues with a side group at the C-5 position of the monolignol ring, and said transformed plant having an untransformed (wild-type) natural plant whose lignin contains no syringyl residues.

A still further aspect of the invention relates to a method of producing cellulose-containing pulp useful for papermaking and the like, which comprises a lignin-containing biomass derived from a gymnosperm plant to produce pulped mass containing lignin, and removing most of said lignin from said pulped mass, characterized in that said gymnosperm plant is a genetically transformed plant producing lignin containing syringyl residues or other residures with a side group at the C-5 position of the monolignol ring.

As will be appreciated from the above, the present invention is capable of producing transformed gymnosperm plants having a modified lignin content that makes gymnosperm plants more attractive on a commercial and industrial scale.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram showing the basic lignin biosynthetic pathway, the enzyme abbreviations being as described in this application, and the suggested induced pathway(s) being highlighted (the inserted box indicates the numbering in the phenyl ring). Note the three branches of the phenylpropanoid pathway (labeled 1), 2) and 3)): branch 1 yields the monolignol ρ-coumaryl alcohol, present in some angiosperms and gymnosperms; branch 2 yields the monolignol coniferyl alcohol, which is present in both angiosperms and gymnosperms yet is the predominant monolignol in gymnosperms; and branch 3 yields sinapyl alcohol predominant and present only in angiosperms, with very few exceptions.

Figure 2 is a graph showing the mean height growth in 1997 from three different transformed lines derived from two different parental genotypes of F5H-transformed and control (non-transformed) interior spruce somatic

seedlings (note F5H 2d-1 and 2d–2 are two replicate sets of somatic seedlings planted 2 weeks apart);

Figure 3 shows the result of a PCR amplification of a 750 bp arabidopsis F5H fragment using the primer pair cc8/cs278 from 14 Putative Transformed Lines (lanes 1-14); a Non-Transformed I1026 Negative Control (lane 15); a Plasmid only p482-F5H = pCC87Positive Control (lane 16); a Plasmid only pBIC-F5H = pBIC20F5H, Control (lane 17); a Blank, no DNA Negative Control (lane 18); and Molecular Weight (MW) Markers (lane 19); and

Figure 4 shows the genomic nucleotide and amino acid sequences of a known Arabidopsis F5H gene and the F5H enzyme that it encodes (as disclosed in Chapple, WO 97/23599).

BEST MODES FOR CARRYING OUT THE INVENTION

As previously noted, the difficulty in lignin removal in all plants is due to the variety of linkages formed between monolignol precursors during lignin polymerization, which linkages account for lignin polymers being highly heterogeneous. This heterogeneity in lignin and the linkages formed during polymerization have a large influence on the pulping characteristics of wood. For example, the presence of the C-5 methoxylated syringyl residues make hardwood lignin easier to hydrolyze during pulping, while a higher proportion of condensed ρ-hydroxyphenyl residues makes softwood hydrolysis more difficult (Campbell and Sederoff 1996 [6]). This is due in part to the unprotected C-5 group characteristic of ρ-hydroxyphenyl and guaiacyl residues typical of softwoods, accounting for the relatively slower delignification rate of the softwoods (Chiang and Funaoka 1990 [8]).

Any modification of this complex polymer requires an understanding of the metabolic pathway. Fortunately, many steps in the lignin biosynthetic pathway are well understood (Davin et al. 1992[9]). The basic phenylpropanoid pathway is shown in accompanying Figure 1. The pathway begins with the conversion of L-phenylalanine to cinnamic acid, by phenylalanine ammonia-lyase (PAL) followed by the conversion of cinnamate to 4-coumarate by cinnamate 4-hydroxylase (C4H). 4-Coumarate has several potential metabolic fates and these account for pathways to the three monolignol precursors. Thus, 4-coumarate can enter into one of the three monolignol branches of the phenylpropanoid pathway shown in Figure 1 as follows:

Branch 1) Present in both angiosperms and gymnosperms where 4-coumarate is activated to 4-coumaryl-CoA in a reaction catalyzed by 4-coumaryl-CoA ligase (4CL), and reduced by hydroxycinnamyl-CoA reductase (CCR) and cinnamyl alcohol: NAD oxidoreductase (CAD) to 4-hydroxycinnamyl alcohol (ρ -coumaryl alcohol), the first of the three monomeric lignin precursors;

Branch 2) Present in both angiosperms and gymnosperms where 4-coumerate is either: A) 3-hydroxylated and 3-O-methylated to form ferulic acid, followed by activation by 4CL, and reduced by CCR and CAD to yield 3-methoxy-4-hydroxycinnamyl alcohol (coniferyl alcohol), the major lignin precursor in conifers; or B) activated to 4-coumarly-CoA which is subsequently 3-hydroxylated and 3-O-methylated to form feruloyl-CoA, which is then reduced by both CCR and CAD to yield 3-methoxy-4-hydroxycinnamyl alcohol; and

Branch 3) Present only in angiosperms where 4-coumarate is modified as in branch 2); however, either: A) ferulic acid undergoes a ring-hydroxylation by ferulate 5-hydroxylase (F5H) and O-methylation by an O-methyltransferase (OMT) to generate sinapic acid, which is reduced to yield sinapyl alcohol, the source of the syringyl residues typical in angiosperms; or B) proceeds through branch 2 to coniferaldehyde and then to 5-hydroxyconiferaldehyde to sinapaldehyde to sinapyl alcohol; or C) proceeds through branch 2 to coniferaldehyde and then to 5-hydroxyconiferyl alcohol to sinapyl alcohol.

The present invention involves the genetic engineering of gymnosperms to introduce one or more functional genes encoding one or more enzymes that results in a modification of the lignin composition of a gymnosperm plant that makes the gymnosperm plant or plant products more commercially desirable. The modification of gymnosperms with genes for any of the enzymes capable of affecting the phenylpropanoid pathway is within the scope of the invention, provided such genes modify the lignin composition of gymnosperm plants to make the plants more commercially desirable. Preferably, the transgene creates a Branch 3 metabolic pathway, or other residues with a side group at the C-5 position of the monolignol ring, and most preferably one of the genes encodes ferulate 5-hydroxylase (F5H). As noted above, this enzyme is thought to be absent in most gymnosperms (with few exceptions) and is one of the key enzymes missing in conifers which accounts for the difference between angiosperm and gymnosperm lignin (Campbell and Sederoff 1996 [6]). The exceptions are very few as previously noted and include reports of syringyl lignin in the non-coniferales gymnosperm species *Podocarpus* and in some species of the *Gnetales*. These exceptions do not, however, detract from the invention as the vast

number of gymnosperms do not produce syringyl lignin and these exceptions are mentioned in a very minor way, if at all in the literature on the subject.

The inventors have been successful in expressing the F5H gene in spruce (a gymnosperm) and have transformed lines containing this transgene in conjunction with other transgenes in the lignin biosynthetic pathway. Since the inventors have demonstrated expression of the F5H gene in spruce, they believe that its expression in other gymnosperm species is predictable since this clearly shows that not only the F5H gene can be expressed in gymnosperms, but also that its expression can modify lignin in plants which do not contain a pathway for syringyl lignin.

Although not conclusive, support that this single enzyme (F5H), either alone or in conjunction with other enzymes, will alter gymnosperm lignin comes from the fact that mutants of the angiosperm *Arabidopsis* which lack this enzyme produce lignin similar in composition to gymnosperms (Chapple et al. 1992), suggesting the lack of this one enzyme, alone or in conjunction with other enzymes, can account for the difference in lignin composition in an angiosperm where a branch of the phenylpropanoid pathway to guaiacyl-containing lignin already exists.

As noted above, the F5H gene is known and described, e.g. in PCT publication WO 97/23599 on July 3, 1997. The disclosure of this publication is specifically incorporated herein by reference. For convenience, the nucleotide sequence of the F5H gene from *Arabidopsis* and the amino acid sequence of the F5H enzyme is shown in Figure 4 of the accompanying drawings.

The F5H gene can be obtained from an angiosperm species, e.g. *Arabidopsis thaliana,* DNA either by polymerase chain reaction (PCR) using

primers designed to the 5' and 3' ends of the published F5H sequence in Figure 4, or by plasmid rescue of the *fah*1 mutant and complementation as was done by Meyer et al. (1996[15]). The PCR amplified product can then be used to identify the native gene from either a genomic or cDNA library and the gene can be subsequently cloned by standard gene cloning techniques. The isolation of the gene by PCR or from the *fah*1 mutant is believed to be within the competence of any person skilled in the art, so that further explanation is unnecessary. Similar techniques can and have been used to isolate other genes in the lignin biosynthetic pathway which can be used in conjunction with an F5H gene to modify lignin in gymnosperms.

Several constructs of the F5H gene were obtained as explained in the PCT publication mentioned above. These constructs include either genomic and cDNA F5H genes controlled by a cauliflower mosaic virus (CaMV) 35S or cinnimate 4-hydroxylase (C4H) promoter, as well as a C4H-GUS construct to test the expression pattern of the C4H promoter, as well as an OMT construct used in conjunction with F5H and a construct containing an F5H-OMT translational fusion. These and other constructs used in this invention are listed below:

pGA482-F5H = pCC87	a pGA482-based vector containing a CaMV 35S-genomic F5H construct;
pBIC-F5H = pBIC20-F5H	a pBIC20-based vector containing an 18kb genomic fragment containing both the F5H promoter and coding region;
pCC98	a pBI121-based vector containing a CaMV 35S-cDNA F5H construct;

pCC223	a pBI101-based vector containing a C4H-GUS construct for xylem directed expression of GUS;
pC4H-F5H = pCC153	a pGA482-based vector containing a C4H-genomic F5H construct; and
pCC99	a pGA482-based vector containing a double-CaMV 35S-genomic F5H construct.
parabOMT	a pUC based vector containing a CaMV 35S-OMT construct used for co-blasting with pCC99.
pF5H-OMT	a pBINPLUS derived vector containing a double-CaMV 35S-F5H-OMT translational fusion.

Starting materials for such vectors are in common use for the construction of plant transformation vectors and are generally available around the world from various labs. The pBI- series is commercially available from Clontech. The pGA482 vector is described in 1987 Methods Enzymol 153:292-305 and is widely used for plant transformation. The pBIC20is a binary cosmid vector described by Meyer et al. 1996, in Genome Mapping in Plants, ed. Paterson, A.H. (Landis Biochemical Press, Austin, TX). Construction of the pGA482-F5H and pBIC20-F5H plasmids are detailed in Meyer et al., 1996, PNAS, 93.6869-6874 and both are available from that source (Chapple). The other F5H and OMT constructs were made using similar techniques.

As seen in the PCT publication mentioned above, the CaMV 35S constructs have been used successfully to modify lignin content in both *Arabidopsis* and tobacco and were included in the present invention to give ectopic expression of the F5H gene in spruce. The C4H promoter constructs should

direct expression to the xylem, the principal target tissue for lignin modification. Because the C4H promoter was isolated from an *Arabidopsis* C4H gene, its expression - as well as the expression of the native C4H gene - in gymnosperms was previously unknown. The OMT constructs were included to ensure, if needed, the O-methylation of the 5-hydroxylated branch 2 intermediates.

To initiate transformation experiments, the plasmids were transformed into *E. coli* and were subsequently purified by CsCl gradient centrifugation. Each plasmid was checked by restriction digest to confirm its identity. Standard procedures were used for coating gold particles with the plasmids and for microprojectile bombardment of spruce somatic embryos. Regeneration of transformed spruce callus was done on a very low level of kanamycin (2–5μg/ml) and embryo maturation was done using routine protocols for spruce.

Over 10,000 spruce embryos were blasted with the various constructs and over 500 transformed seedlings from over 50 transformed lines have been planted in the greenhouse. No abnormal phenotypes or altered growth patterns have been detected in any of the transformed embryogenic callus lines or seedlings. The results of these experiments are summarized in Table 1 below.

Table 1

Information regarding tested constructs

CONSTRUCT	# KANAMYCIN RESISTANT LINES	# CONFIRMED PCR POSITIVE LINES	# CONFIRMED SOUTHERN POSITIVE LINES	# CONFIRMED NORTHERN POSITIVE LINES	# OF LINES WITH SEEDLINGS REGENERATED
pCC87 35S-gF5H	16	16	8	5	18
pBIC20-F5H F5H-gF5H	6	Inconclusive	Inconclusive	Inconclusive	6
pCC98 35S-cF5H	5	2 – Inconclusive	2	nd	1
pCC223 C4H-GUS	6	5	3	ND	5
pCC153 C4H-gF5H	16	10	6	2	12
pCC99 2X 35S-gF5H	20	19	3	17	18
pCC99+ parabOMT	11	Nd	nd	nd	0
Total	80	47	22	24	60

Figure 2 of the accompanying drawings shows the mean height growth of three different F5H transformed lines from two different parental embryogenic genotypes compared with non-transformed somatic seedlings over the growing season. Transformed line I1026 2d is represented by two lots of somatic seedlings planted two weeks apart.

A set of six nested primers for the F5H gene were obtained and tested for amplification of the F5H gene from pCC87.The primer pair consisting of cc8 and cs278 were used to amplify a band of approximately 750bp from the genomic F5H gene.

Figure 3 confirms integration by PCR of the Arabidopsis F5H in 14 different putative F5H transformed embryogenic callus lines (lanes 1–14). A band of approximately 300bp in all lanes including the blank (lane 18) and the non-transformed I1026 control (lane 15), suggests that this fragment is a non-specific amplification product. The band of interest, a 750bp amplification product, is very prominent as expected in the pCC87 plasmid only lane (lane 16) and is absent from both the blank and the non-transformed control. Note the presence of a 750bp band in DNA samples from 10 transformed lines (lanes 1,3,6,7,8,9,11,12,13,14) including the three transformed lines which have somatic seedlings in the greenhouse. The absence of the 750bp band in the remaining putative transformed lines could indicate that these lines are non-transformed escapes, or that the DNA preparation from these lines was poor. This later suggestion is supported by the lack of other background bands in these lanes (lanes 2,4,5,10).

Northern blot analyses have confirmed strong expression of the F5H gene in spruce and Southern blot analysis of transformed lines have conclusively confirmed the PCR results for integration of the inserted DNA into the spruce genome (Table 1).

REFERENCES

1. Bao et al. 1993. Science 260: 672-676;
2. Bate et al. 1994. Proc Natl Acad Sci USA 84:980-984;
3. Bell-LeLong et al. 1997. Plant Physiol, Plant Physiol 113(3):729.
4. Bugos et al. 1991. Plant Mol Biol 17:1203-1215;
5. Campbell and Ellis. 1992. Planta 186:409-417;
6. Campbell and Sederoff. 1996. Plant Physiol 110:3-13;
7. Chapple et al. 1992. Plant Cell 4:1413-1424;
8. Chiang and Funaoka. 1990. Holzforschung 44:147-155.
9. Davin et al. 1992. Rec Adv Phytochem 26: 325-376;
10. Dwivedi et al. 1994. Plant Mol Biol 26:61-71;
11. Ellis et al. 1993. Bio/Technology 11:84-89;
12. Halpin et al. 1994. Plant J 6:339-350;
13. Iiyama and Wallis. 1990. J Sci Food Agric 51: 145-161;
14. Langrimini et al. 199 Plant Cell 2:7-8;
15. Meyer et al. 1996. PNAS 93:6869-6874;
16. Ni WT, Paiva NL and Dixon RA. 1994 Transgenic Res 3(2):120-126
17. Whetton, R.W., MacKay, J.J., and Sederoff, R.R. 1998 Ann. Rev. Plant Physiol. Plant Mol. Biol. 49:585-609.

The disclosures of all of the above publications are specifically incorporated herein by reference.